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Heptatriacontanol and Phenolic Compounds from *Halochris hispida*

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Abstract – The phytochemical investigation of *Halocharis hispida* revealed the presence of 1-heptatriacontanol, β-sitosterol, β-sitosterol-3-*O*-glucoside, kaempferol, vitexin and isorhamnetin-3-*O*-galactoside in addition to vanillic, ferulic, isoferulic, syringic and caffeic acids. The different isolated compounds were identified by different physical, chemical, chromatographic and/or spectral methods.
Key words – *Halocharis hispida*, Chenopodiaceae, paraffinic alcohol, phytosterols, flavonoids, phenolic acids.

Introduction

Family Chenopodiacae (Goosefoot Family) encompasses 102 genera and 1400 species of wide distribution (Bailey, 1975). Different members of the family were reputed for their medicinal uses, as antifungal, anthelmentic, purgative, antispasmodic, diaphoretic, cough suppressant, emenagogue, treatment of asthma and migraine, sores, eczema and erysipelas and intestinal ulceration (Watt and Breyer-Brandwijk, 1962). From the phytochemical point of view, betalains, alkaloids, phenolic acids, saponins, and glycosides were reported as the main active constituents of the family (Gibbs, 1974).

H. hispida is an annual desert plant sporadically growing in the wades and Riyadh area, in Saudi Arabia and reputed among the Bedwins as antiinflamatory, demulcent, antispasmodic and effective for the treatment of wounds. Reviewing the current literature, the fungicidal antibiotic halocharine was reported in the aerial parts of the plant (Bondorenko *et al.*, 1970). This pursued further investigation of the plant from the phytochemical point of view.

Experimental

General – Mp. (uncorr.); UV (MeOH); ^{1}H-NMR, 600 MHz and ^{13}C-NMR, 150 MHz, TMS as int. standard, solvent DMSO-d_6, δ ppm; MS, positive FAB-MS (*m/z*); TLC: Si gel 60 F_{254} plates; EtOAc-CH_3OH-H_2O (10:1.5:1), $AlCl_3$ spray **S1;** *n*-hexane-EtOAc (3:1), vanillin sulfuric acid spray **S2**, $CHCl_3$-CH_3OH (9:1) $AlCl_3$ spray **S3,** EtOAc-toluene-formic acid (10:9:1) $FeCl_3$ spray **S4**. Acid hydrolysis: alcoholic solution of compounds **4** and **6** (5mg each) was refluxed on boiling water bath for 1h. The excess acid was neutralized with Ag_2O, alcohol evaporated and aglycone was extracted with EtOAc and examined by TLC and the sugar was examined by PC: Whatman filter paper No. 1, butanol-benzene-pyridine-water (4:1:3:3) solvent (overnight), aniline phthalate spray **S5**.

Plant material – *Halocharis hispida* (C.A. Meg) Bunge, Chenopodiaceae, was collected from Saudi Arabia desert, Riyadh area in the flowering stage in 1995. Dr. Sultan Ul-Abidin, taxonomist, Faculty of Pharmacy, KSU, confirmed the identity of the plant. A voucher specimen was deposited at the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Extraction and fractionation – The dried powdered aerial parts (1 kg) of *H. hispida* were percolated with 70% ethanol (8 L). The extract was concentrated *in vacuo* to ca. 500 ml, partitioned with $CHCl_3$ followed with EtOAc (0.31%). The $CHCl_3$ extract was partitioned between *n*-hexane (0.77%) and 90% EtOH (0.92%). The aqueous mother liquor was completely evaporated *in vacuo* and the residue was partitioned as CH_3OH soluble (5.23%) and insoluble (12%) fractions.

Isolation – The residue of *n*-hexane fraction was dissolved in petroleum ether, passed over charcoal column (20g) and elution was continued with petroleum ether, then CH_2Cl_2 followed by EtOAc (500 ml, each). CH_2Cl_2 fraction (5.5 g) was adsorbed on 10 g of Si gel and loaded on Si gel column 150 g.

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and the compounds were gradually eluted with a mixture of *n*-hexane-ethyl acetate to afford compound **1** (227 mg) and compound **2** (991 mg) eluted with 20% and 30% EtOAc/hexane, respectively.

Ethanol fraction (8 g) was adsorbed on 16 g of Si gel and loaded on Si gel column 200 g, and compounds were gradually eluted with mixture of $CHCl_3$-CH_3OH to afford compound **3** (45 mg) and compound **4** (58 mg) eluted with 4% and 6% $CH_3OH/CHCl_3$, respectively.

The ethyl acetate fraction 2.5 g was adsorbed on 6 g of Si gel and loaded on Si gel column 80 g. Elution was done isocratic with EtOAc-CH_3OH-H_2O (100:10:5). This column afforded compounds **3** and **4** as minor components followed by compounds **5** (42 mg) and mixture of **5** and **6** (95 mg). The latter mixture was loaded on Si RPC18, 30 g column and elution was done with water-methanol (7:3) to afford **6** (20 mg) and **5** (48 mg).

An aliquot of the EtOAc extract was analyzed for the phenolic acids content using silica gel 60 (Merck), EtOAc-toluene-formic acid (10:9:1) solvent, $FeCl_3$ spray.

Results

Compound 1 – White plates, mp. 91°, IR ν_{max} (KBr): 3350 (br) (O-H stretching), 2900 (C-H stretching), 1060 (C-O primary alcohol stretching), 730-720 (CH_2 rocking; bi-forked band) cm^{-1}. FAB-MS *m/z* (rel. int.): 559 ($M+Na^+$), 575 ($M+K^+$), 536 (M^+), 518 (M^+-H_2O), 503 ($M^+-H_2O-CH_3$), 31 (CH_2OH), 69 [$(CH2)_4$-CH]. ^{1}H-NMR ($CDCl_3$, δ ppm): 3.62 (t, 2H, H-1), 1.55 (m, 2H, H-2), 1.26 (m, 68H), 0.86 (t, 3H, H-37). ^{13}C-NMR ($CDCl_3$, δ ppm): 63.11 (C-1), 32.87 (C-2), 31.94 (C-3), 29.70-29.62 (C-4 - C-32), 29.44 (C-33), 29.36 (C-34), 25.77 (C-35), 22.68 (C-36), 14.07 (C-37).

Compound 2 – White needles, mp. 137°, IR ν_{max} (KBr): 3450, 2920, 1650, 1460, 1380, 1050, 980 cm^{-1}

Compound 3 – Yellow needles, mp. 278°, UV (Table 2), ^{1}H-NMR (δ ppm): 8.07 (d, *J*=8.69 Hz, H-2', H-6'), 6.89 (d, *J*=8.79 Hz, H-3', H-5'), 6.39 (d, *J*=1.47 Hz, H-8), 6.17 (d, *J*=2.19Hz, H-6).

Compound 4 – White rosettes, mp. 289°, IR ν_{max} (KBr): 3450, 2920, 1650, 1460, 1380, 1265, 1100-1000 (broad), 800 cm^{-1}.

Compound 5 – Yellowish needles, mp. 265°. UV (Table 2), FAB-MS *m/z* (rel. int.): 525 (M^++1+ glycerol), 433 (M^++1), $C_{21}H_{20}O_{10}$. ^{1}H-NMR (δ ppm): 8.2 (2H, d, *J*=9.0 Hz, H-2', H-6'), 6.89 (2H, d, *J*=8.4 Hz, H-3', H-5'), 6.77 (H-3), 6.27 (H-6), 4.67 (d, *J*= 9.6 Hz, H-1"), 3.81 (t, H-2"), 3.25 (m, H-3"), 3.35 (m, H-4"), 3.22 (m, H-5"), 3.73 (d, *J*=12 Hz, H-6"α), 3.51 (dd, *J*=6, 12 Hz, H-6"β). ^{13}C-NMR (δ ppm): 163.9 (C-2), 102.56 (C-3), 182.05 (C-4), 160.07 (C-5), 97.99 (C-6), 162.31 (C-7), 104.60 (C-8), 156.03 (C-9), 104.06 (C-10), 121.67 (C-1'), 128.57 (C-2', C-6'), 115.73 (C-3', C-5'), 160.94 (C-4'), 73.35 (C-1"), 70.70 (C-2"), 78.44 (C-3"), 70.39 (C-4"), 81.80 (C-5"), 61.15 (C-6"). HMBC: δ 6.27 (H-6) and δ 104.60 (C-8) & δ 104.06 (C-10); δ 6.77 (H-3) and δ 104.06 (C-10) & 163.9 (C-2); δ 8.2 (H2', H-6') and δ 163.9 (C-2) & 160.94 (C-4'); δ 6.89 (H-3', H-5') and δ 121.67 (C-1'); δ 4.67 (H-1") and δ 162.31 (C-7) & 156.03 (C-9).

Table 1. Phenolic acid contents in the ethyl acetate fraction

Spot R_f	UV colour	$FeCl_3$	Identification against authentic
0.55	Blue	Reddish brown	Vanellic acid
0.54	Light blue	Reddish brown	Ferulic acid
0.51	Light blue	Reddish brown	Isoferulic acid
0.47	Blue	Reddish brown	Syrengic acid
0.45	Light blue	Dull green	Caffeic acid

Table 2. UV data of the flavonoids

Reagent/compd.	Keampferol	Vitexin	Isorhamnetin 3-*O*-galactoside
CH_3OH	265, 371	253, 268,338	256, 270^{sh}, 357
$NaOCH_3$	263, 285, 359^{sh}, 451	279, 328, 395	274, 328^{sh}, 415
$AlCl_3$	261, 300^{sh}, 364, 426	278, 305, 348, 385	270, 299^{sh}, 368, 408
HCl	261, 300^{sh}, 346^{sh}, 427	278, 305, 348, 383	269, 300^{sh}, 357, 404
NaOAc	272, 322^{sh}, 384	278, 300^{sh}, 379	273, 317, 390
H_3BO_3	257, 314^{sh}, 369	253, 329^{sh}, 345	257, 267^{sh}, 361

Compound 6 – Yellowish needles, mp. 235°. UV (Table 2), FAB-MS *m/z* (rel. int.): 570 (M+glycerol), 479 (M+1), $C_{22}H_{22}O_{12}$, 317 (M+1-hexose). ^{1}H-NMR (δ ppm): 6.17 (d, *J*=1.8 Hz, H-8), 6.42 (d, *J*=2.4Hz, H-6'), 7.92 (d, *J*=2.4 Hz, H-2'), 6.69 (d, *J*=8.4 Hz, H-5'), 7.49 (dd, *J*=1.8, 8.4 Hz, H-6'), 3.93 (3H, s, OCH_3), 5.73 (d, *J*=7.8 Hz, H-1"), 3.34-4.69 (m, H-2 - H-5), 4.67 (2H, d, *J*=10.2 Hz, H-6"). ^{13}C-NMR (δ ppm): 156.68 (C-2), 133.82 (C-3), 177.07 (C-4), 161.70 (C-5), 99.50 (C-6), 164.95 (C-7), 94.51 (C-8), 157.01 (C-9), 104.58 (C-10), 121.73 (C-1'), 113.97 (C-2'), 149.91 (C-3'), 147.64 (C-4'), 115.83 (C-5'), 122.73 (C-6'), 56.6 (OCH_3), 102.30 (C-1"), 71.87 (C-2"), 76.32 (C-3"), 68.55 (C-4"), 73.69 (C-5"), 60.93 (C-6").

Discussion

The total EtOH extract of *H. hispida* was fractionated between different solvents into different fractions *viz.* $CHCl_3$, EtOAc, CH_3OH soluble and insoluble. The first was again fractionated between *n*-hexane and EtOH (90%). Phytochemical screening of the different fractions revealed that the *n*-hexane contained sterols and/or triterpenes, EtOH (90%) contained flavonoids, sterols and/or triterpenes glycosides, phenolic acids, EtOAc contained phenolic acids and flavonoids and the methanol soluble fraction contained flavonoids, quaternary ammonium compounds (Balbaa *et al.*, 1981).

Column chromatography of the partially purified *n*-hexane fraction afforded two compounds. Compound **1** gave negative tests for steroids, triterpenes and unsaturation. IR spectrum of **1** showed broad absorption peak at 3350 cm^{-1} indicating hydroxyl group, CH_2 stretching (2900), C-O primary alcohol stretching (1060) and for CH_3 at 725 cm^{-1}. This suggests paraffenic alcohol structure (Brown, 1995). MS of **1** showed molecular peak at *m/z* 536 calculated for $C_{37}H_{76}O$, 518 (M-H_2O), 503 (M-H_2O-CH_3), 69 (C_4H_8-CH), 31 (CH_2-OH) fragmentation pattern characteristic for long chain saturated alcohol (Barker, 1999). ^{13}C-NMR and DEPT experiment showed only one CH_3 carbon at δ 14.07 and CH_2 with oxygen function at δ 63.11. The other CH_2 carbon atoms resonated at δ 32.87-22.68. ^{1}H-NMR chemical shift signals were in full agreement with those reported for primary alcohol (Brown, 1995). ^{1}H-^{1}H COSY experiment unambiguously confirmed the previous conclusion, where cross peak signal appeared between the signals at δ 0.87 (CH_3) and at δ 1.26 (for 68 protons). The latter also correlated with the 2Hs at δ 1.55 (β-carbon protons). These β-protons were in turn correlated with the 2'Hs at δ 3.62 (α-carbon protons-CH_2OH). Accordingly, compound **1** should be heptatriacontanol. This compound was previously detected in the bark of *Erythrina stricta* by glc (Singh *et al.*, 1981).

Compound **2** was proved to be β-sitosterol by co-chromatography against reference sample **S2**, colour reactions and comparison of its mp and IR with those reported (Balbaa *et al.*, 1981).

Column chromatography of the EtOH fraction afforded two compounds. Compound **3** was proved to be kaempferol as concluded from its ^{1}H-NMR spectrum and comparison of its UV spectra and mp. with the reported data as well as co-chromatography with reference sample, **S3** (Mabry *et al.*, 1970; Markham, 1982; Gohar *et al.*, 2000). Compound **4** was concluded to be β-sitosterol 3-*O*-glucoside. The compound gave positive reactions for steroid and/or triterpene glycosides. Acid hydrolysis of the compound proved the presence of β-sitosterol aglycone (co-chromatography with reference sample **S2**) and glucose as the sugar moiety **S5**. Moreover the IR spectrum of the compound was superimposed with that of reference sample.

TLC of the EtOAc fraction **S1** revealed the presence of two major flavonoids. Column chromatography of this fraction on silica gel followed by RPC18 afforded two major flavonoids, **5** and **6**. The identity of compound **5** was proved to be vitexin as deduced from its ^{1}H-NMR spectrum. A typical AB-system splitting patterns were shown with two doublets at δ 8.2 (*J*=9.0 Hz) and δ 6.89 (*J*=8.4 Hz) for H-2', H-6' and H-3', H-5', respectively. The chemical shifts of the corresponding carbons confirmed this system. Two proton singlets at δ 6.77 and δ 6.27 were assigned for H-3 and H-6 (Mabry *et al.*, 1970; Markham, 1982). The presence of 6-oxygenated aliphatic carbon signals, for the sugar, in the range of 61-85 ppm indicated that the sugar moiety was hexose and linked to the aglycone skeleton by C-C linkage (Agrawal and Bansal, 1989). The downfield shift of carbon 8 (7-12 ppm, from 94.2 to 104.6 ppm) proved that the sugar attachment should be at that site (Markham and Chari, 1982). The identity of vitexin rather than isovitexin was unambiguously confirmed from the HMBC experiment. H-6 was correlated with C-8 & C-10, H-3 with C-2 & C-10, H-2 and H-6 with C-2 & C-4', H-3' and H-5' with C-1' and H-1" with C-7 & C-9 (see Results). Moreover

the carbon-13 nmr data of the compound was in full agreement with those reported for vitexin (Markham and Chari, 1982). The identity of **6** was proved to be isorhamnetin-3-*O*-galactoside as deduced from comparison of its physical and chemical data with the published (Mabry *et al.,* 1970; Markham *et al.*, 1978; Sarkar and Friedrich, 1980; Markham, 1982; Chaurasia and Wichtl, 1987). Moreover, acid hydrolysis of **6** proved the presence of isorhamnetin aglycone (co-chromatography with reference sample **S3**) and galactose as the sugar moiety **S5** (traveled distance, 5 cm for galactose and 6 cm for glucose).

TLC of the EtOAc as well as the EtOH fractions **S1** revealed different blue fluorescent spots relatively with appreciable amount in the EtOAc fraction. Analysis of this fraction for its phenolic acid content against reference **S4** samples revealed the presence of vanillic, ferulic, isoferulic syringic and caffeic acids.

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